DISCOVERY

Effect of *Andrographis paniculata* on carbon tetrachloride-induced acute liver injury in the rat

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ABSTRACT

The effect of a standardised extract of *Andrographis paniculata* (*A. paniculata*) on acute liver damage induced by carbon tetrachloride (CCl_4) was investigated. Rats were given CCl_4 once daily for a week, either alone or in combination with *A. paniculata* at doses of 50, 100, or 200 mg/kg or silymarin at 25 mg/kg. To evaluate liver damage, hepatic histology, DNA ploidy tests and serum liver enzyme activity were used. The results demonstrated that serum alanine aminotransferase and aspartate aminotransferase activities were significantly and noticeably increased by CCl_4. On the liver histology, there was massive vacuolar degeneration, acidophilic hepatocytes and inflammatory cellular infiltration. Additionally, sections stained with the Feulgen stain for DNA studies showed that the CCl_4-treated group had a high proliferating index and a significantly lower DNA content (hypoploidy) than the vehicle control group. *A. paniculata* administered to CCl_4-treated rats had a strong liver protective effect that led to significant dose-dependent decreases in the serum aminotransferases. Additionally, its dose-dependently lessened the histological alterations caused by CCl_4, with the highest dose of the extract nearly returning the liver tissue to normal. The highest dose of the extract produced DNA values that were comparable to the vehicle control values when *A. paniculata* and CCl_4 were administered. These findings show that *A. paniculata* protects against acute liver damage produced by CCl_4, reducing the extent of histological liver damage, increases in serum transaminases and DNA changes caused by the hepatic toxicant.

Keywords: *Andrographis paniculate*, carbon tetrachloride, liver injury, liver enzymes, DNA ploidy, hepatoprotective

1. INTRODUCTION

The liver is the largest organ in the body and is where most of the body's protein, lipid and glucose metabolism takes place. Additionally, the liver plays a critical role in the detoxification of a variety of pharmaceuticals, environmental toxins and xenobiotics that, through the active metabolites they produce, such as electrophilic compounds or oxidant free radicals, may contribute to or hasten the deterioration of liver cells (Österreicher, 2012). These reactive oxygen and nitrogen species, free radicals, and other reactive molecules have the capacity to damage mitochondria, which causes hepatocellular necrosis and energy

depletion. They can also deplete cellular antioxidants such reduced glutathione, inactivate enzymes, damage cellular proteins, lipids and nucleic acids (Kaplowitz, 2004).

The use of herbal treatments and other natural remedies for the treatment of liver problems is gaining popularity across the globe. The "King of Bitters" is another name for the traditional medicinal plant *Andrographis paniculata* (Burm. f.) Nees (family *Acanthaceae*). In Southeast Asia, this annual herbaceous plant is widely farmed. The herb is frequently used to treat a variety of human illnesses, including malaria, diarrhoea, dyspepsia, respiratory tract infections, the common cold and parasitic infections. It is frequently used alone or in combination with other therapeutic herbs as a powder, infusion, or decoction. Additionally, standardised *A. paniculata* extract preparations that are commercially available are used in a number of countries to treat the common cold (Hancke et al., 1995; Hu et al., 2017).

Important pharmacological activities of *A. paniculata* include anti-inflammatory, analgesic, antipyretic, immunomodulating, antidiabetic, as well as hepatoprotective actions (Mishra et al., 2007; Hossain et al., 2021). The therapeutic actions of *A. paniculata* extracts are attributed to a number of the active components they contain. Extracts of *A. paniculata* have yielded diterpenoids, diterpene glycosides, flavonoids, flavonoid glycosides and lactones (Tang and Eisenbrand, 1992). The plant also included flavonoids such apigenin, onsilyin and 3,4-dicaffeolyquinic (Raman et al., 2022). The main diterpenoids identified from *A. paniculata* are andrographolide, neo andrographolide, 14-deoxyandrographolide, andrographiside, 14-deoxyandrographiside, 14-deoxy-11,12-didehydroandrographolide and 14-deoxy-11, 12-didehydro-andrographiside (Mishra et al., 2007). The most prevalent compound in plant leaves, andrographolide, has been shown to inhibit nuclear factor kappa-B (Hidalgo et al., 2005; Bao et al., 2009), cyclooxygenase-2 (Liu et al., 2007), inducible nitric oxide synthetase (Chiou et al., 2000) and other inflammatory mediators in inflammatory cells in vitro.

In this investigation, the capacity of a standardised commercial *A. paniculata* extract to protect the liver against acute carbon tetrachloride-induced injury in rats was investigated. The toxicant is a common industrial solvent that can cause significant acute liver damage; hence it is employed to in the search of new therapies for liver disorders (Recknagel et al., 1989).

2. MATERIALS AND METHODS

Animals

Sprague-Dawley rats of either sex weighing 150–160 g was used for the experiments. Animals were kept in controlled environments with free access to tap water and conventional rat chew. The National Research Centre's Ethics Committee and the American National Institutes of Health's Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) were followed when conducting the experiments. In equal groups of six rats each, the investigation was conducted.

Drugs and chemicals

The plant *A. paniculata* 4% standardised commercial extract was utilised (Remdex: Pharmavite Corp, Northridge, Calif). To obtain the necessary doses, the extract was dissolved in physiological saline. Carbon tertrachloride was obtained from Al-Gomhoria Chemicals Co. (Egypt). The doses of *A. paniculata* used in the study were based on the daily dose for humans after being converted to that for rats using conversion tables developed by Paget and Barnes, (1964). The dose of CCl_4 used in the study was based on earlier research (Abdel-Salam et al., 2013).

Experimental groups

Rats were randomly allocated into six groups, each consisting of 6 animals. Rats were gavaged with CCl_4-olive oil (1:1, v/v) at a dose of 2.8 ml/kg for one week in order to induce acute liver injury. To sustain liver damage in rats three days after the first CCl_4 injection, rats were given a dose of CCl_4 that was cut in half (1.4 mg/kg) (Abdel-Salam et al., 2013). Rats were given either the vehicle, *A. paniculata* at doses of 50, 100, or 200 mg/kg, or silymarin at a dose of 25 mg/kg once daily orally for a week starting on the first day of CCl_4 injection.

The following groups were studied:

Group 1 (normal control) received the vehicle (olive oil).
Group 2: Received CCl_4/olive oil and served as positive control.
Group 3: Received CCl_4/olive oil + *A. paniculata* 50 mg/kg.
Group 4: Received CCl_4/olive oil + *A. paniculata* 100 mg/kg.
Group 5: Received CCl_4/olive oil + *A. paniculata* 200 mg/kg.

Group 6: Received CCl_4/olive oil + silymarin 25 mg/kg.

Throughout the investigation, rats had unrestricted access to food and water. At the conclusion of the experiment, rats were decapitated under light ether anaesthesia and blood samples were taken from the retro-orbital vein plexus.

Serum liver enzymes

The Reitman-Frankel colorimetric transaminase method was used for the determination of the activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum (Crowley, 1967). Commercial kits were purchased from BioMérieux in France.

Liver histopathology

Representative liver samples were properly cleaned in formal saline and then fixed for at least 72 hours in 10% neutral-buffered formal saline. The samples were cleaned in tap water for 30 minutes, dehydrated in progressively higher concentrations of alcohol (70-95% absolute), clarified in xylene and finally embedded in paraffin wax. For histological analysis, serial slices of 5 μm thick were cut and stained with hematoxylin and eosin (Hx & E) (Drury and Walligton, 1980).

DNA ploidy studies

DNA investigations were conducted on sections stained with Feulgen stain and counterstained with Light green (Feulgen and Rosenbeck, 1942). Utilizing Leica Quin 500 image cytometry, DNA analysis was carried out (Pathology Department, NRC). 100–120 cells were chosen at random from each area to measure. Control cells were measured in order to determine the threshold values. The percentage of diploid cells (2C), triploid cells (3C), tetraploid cells (4C) and aneuploid cells (> 5C) are displayed as histograms and tables in the results. Danque et al., (1993) classification of the DNA histogram is used.

Statistical analysis

The data is presented as mean ± SE. The data were statistically analysed using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test. Windows version of GraphPad Prism 6 was the programme utilised (GraphPad Prism Software Inc., San Diego, CA, USA). A probability value of 0.05 or less was determined to be statistically significant.

3. RESULTS

Serum liver enzymes

When compared to the corresponding vehicle control values, the serum activities of ALT and AST were elevated by 113.2% (169.9 ± 9.4 vs. 79.7 ± 4.0 U/l) and 80.7% (222.4 ± 8.8 vs. 115.8 ± 3.1 U/l), respectively, following the administration of CCl_4. *A. paniculata* therapy in rats at dosages of 50, 100, or 200 mg/kg was linked to noticeably reduced serum liver enzyme activity. In the CCl_4 + *A. paniculata*-treated groups, serum ALT dramatically decreased by 33.1%, 42.0% and 41.7% from CCl_4 control value of 169.9 ± 9.4 U/l to 113.7 ± 2.9, 98.5 ± 4.2 and 87.9 ± 1.9 U/l, respectively. There was also a substantial reduction in serum AST activity in the *A. paniculata* treatment group compared to the CCl_4 control group by 34.3%, 38.2% and 47.5% (146.0 ± 4.2, 137.3 ± 4.8 and 116.8 ± 7.6 U/l vs. 222.4 ± 8.8 U/l, respectively).

Additionally, there was a substantial reduction in serum AST activity in the *A. paniculata* treatment group compared to the CCl_4 control group of 34.3%, 38.2% and 47.5% (146.0 ± 4.2, 137.3 ± 4.8 and 116.8 ± 7.6 U/l vs. 222.4 ± 8.8 U/l, respectively). When silymarin was administered to CCl_4-treated rats, serum ALT and AST activity levels decreased by 53.0% and 41.0%, respectively, in comparison to the corresponding CCl_4 control values (79.8 ± 5.7 vs. 169.9 ± 9.4 U/l and 131.3 ± 7.8 vs. 222.4 ± 8.8 U/l) (Figure 1).

Liver histopathology

When CCl_4 was administered, it severely damaged the liver tissue, resulting in extensive vacuolar degeneration and acidophilic hepatocytes with cellular infiltration (Figure 2A). This harmful effect was lessened by *A. paniculata* in a dose-dependent way, as the vacuolar degeneration was slightly lessened with 50 mg/kg *A. paniculata* (Figure 2B) and better results were obtained with 100 mg/kg *A. paniculata* (Figure 2C). Although some cellular infiltrates were still seen at the periphery of the lobules, the best outcomes were seen with *A. paniculata* at 200 mg/kg, where normalisation of liver tissue was obvious (Figure 2D).

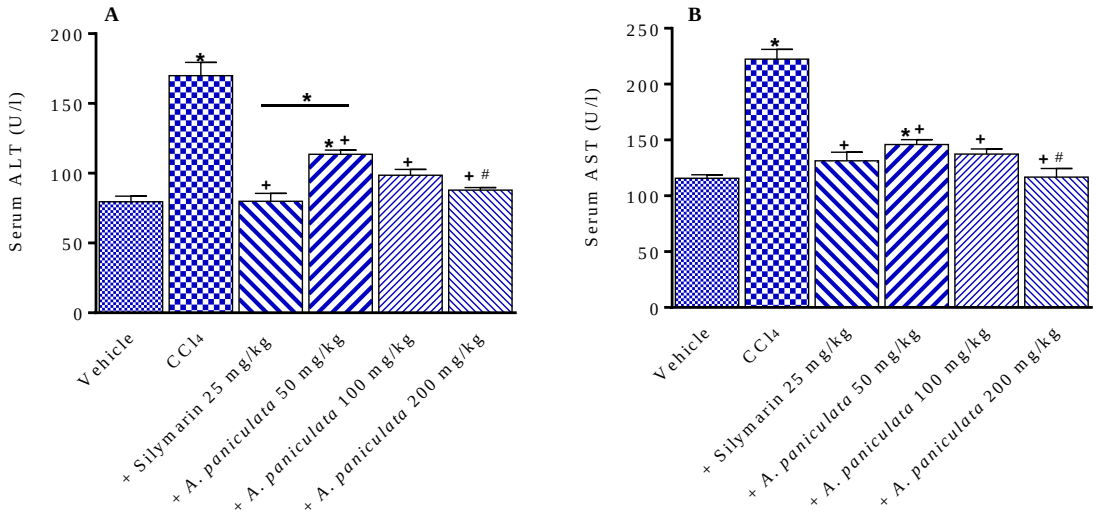

Figure 1 Serum ALT and AST activities in rats treated with CCl_4 alone and after treatment with *A. paniculata*. Results are mean ± SEM. *$p < 0.05$ vs. vehicle control and between different groups as shown in the graph. + $p < 0.05$ vs. CCl_4 control. # $p < 0.05$ vs. CCl_4 + *A. paniculata* 50 mg/kg group.

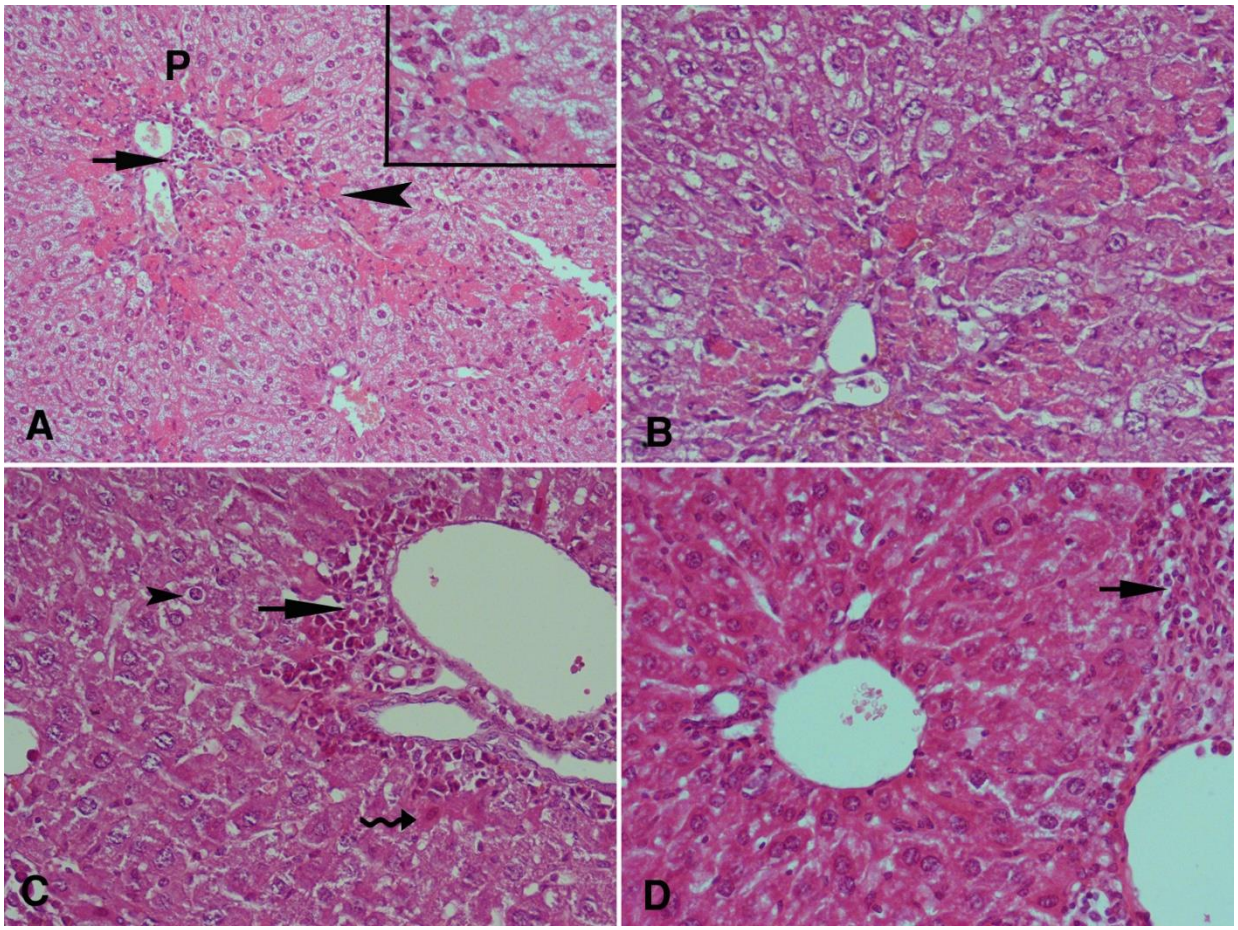

Figure 2 Photomicrographs of liver tissue slices after being exposed to (A) CCl_4 reveals acidophilic hepatocytes all around, cellular infiltration at the portal area (P) and extensive vacuolar degeneration of the majority of hepatocytes (seen in the upper right corner). (B) CCl_4 combined with *A. paniculata* at a dose of 50 mg/kg reveals that vacuolar degeneration is only slightly reduced, but acidophilic hepatocytes are still present. (C) CCl_4 and *A. paniculata* at 100 mg/kg shows that even though vacuolar degeneration significantly decreased, there is cellular infiltration surrounding the main blood vessels (arrow) and only a few acidophilic

hepatocytes (wavy arrow) and apoptotic cells are also present. (D) CCl4 plus *A. paniculata* at 200 mg/kg, shows that hepatic tissue is normalised with the exception of some cellular infiltrates at the periphery of the lobule (arrow).

DNA ploidy study

The Qwine 500 image analyzer was employed in the current study to determine the DNA content. The image analysis system automatically expresses the DNA content of every single cell that is measured and then provides the proportion of each cell out of the total number of cells analysed. Additionally, it divides the cells into four categories: Aneuploid cells (> 5C), proliferative cells (3C), tetraploid cells (4C) and diploid cells (2C). According to Lee et al., (1999), the proliferating cells were further divided into three categories: Low proliferation index (10%), medium proliferation index (10–20%) and high proliferation index (> 20%).

Normal distribution of DNA content in the liver cells of the vehicle control group showed that 3.57% of the examined cells contained DNA (< 1.5C), 89.28% contained diploid DNA value (2C), 5.35% contained (3C) DNA value (low Proliferation Index) and 1.78% of the examined cells at (4C) area (Figure 3 & Table 1). Examination of cells from CCl_4 control group (Figure 3 & Table 2) showed that the cells contained DNA (< 1.5C) were 0.0%, while 6.54% contained DNA value (2C) which means a decrease in DNA content (hypoploidy) compared to the vehicle control. In addition, 26.16% of examined cells contained DNA value (3C) (high proliferating index) and 33.64% of examined cells were in (4C) area. Examination of cells from the group treated with CCl_4 and *A. paniculata* at 50 mg/kg shows that cells containing DNA (< 1.5C) were 0.99%, cells that contained DNA value (2C) were 13.86%, while cells that contained (3C) DNA value were 35.64% (high proliferation index). Moreover, 30.69% of cells were in (4C) area (Figure 3 & Table 3).

In the group treated with CCl_4 and *A. paniculata* at 100 mg/kg, 0.0% of the examined cells contained DNA value (< 1.5 C), only 2.77% of cells contained DNA value (2C), while 14.81% of the examined cells contained (3C) DNA value (medium proliferating index) and 21.29% of the examined cells contained (4C) DNA value (Figure 3 & Table 4). Examination of cells from the group treated with CCl_4 and *A. paniculata* at 200 mg/kg shows that 5.88% of cells contained (< 1.5C) DNA value, 26.89% of cells contained DNA value (2C), 23.52% of cells contained (3C) DNA value (high proliferating index) and 14.96% of cells were in the (4C) area (Figure 3 & Table 5). These findings suggest that, particularly with the high dose of the extract medication, treatment with *A. paniculata* coupled with CCl_4 resulted in DNA values equivalent to the vehicle control values, while group treated with CCl_4 showed lower DNA values (hypoploidy).

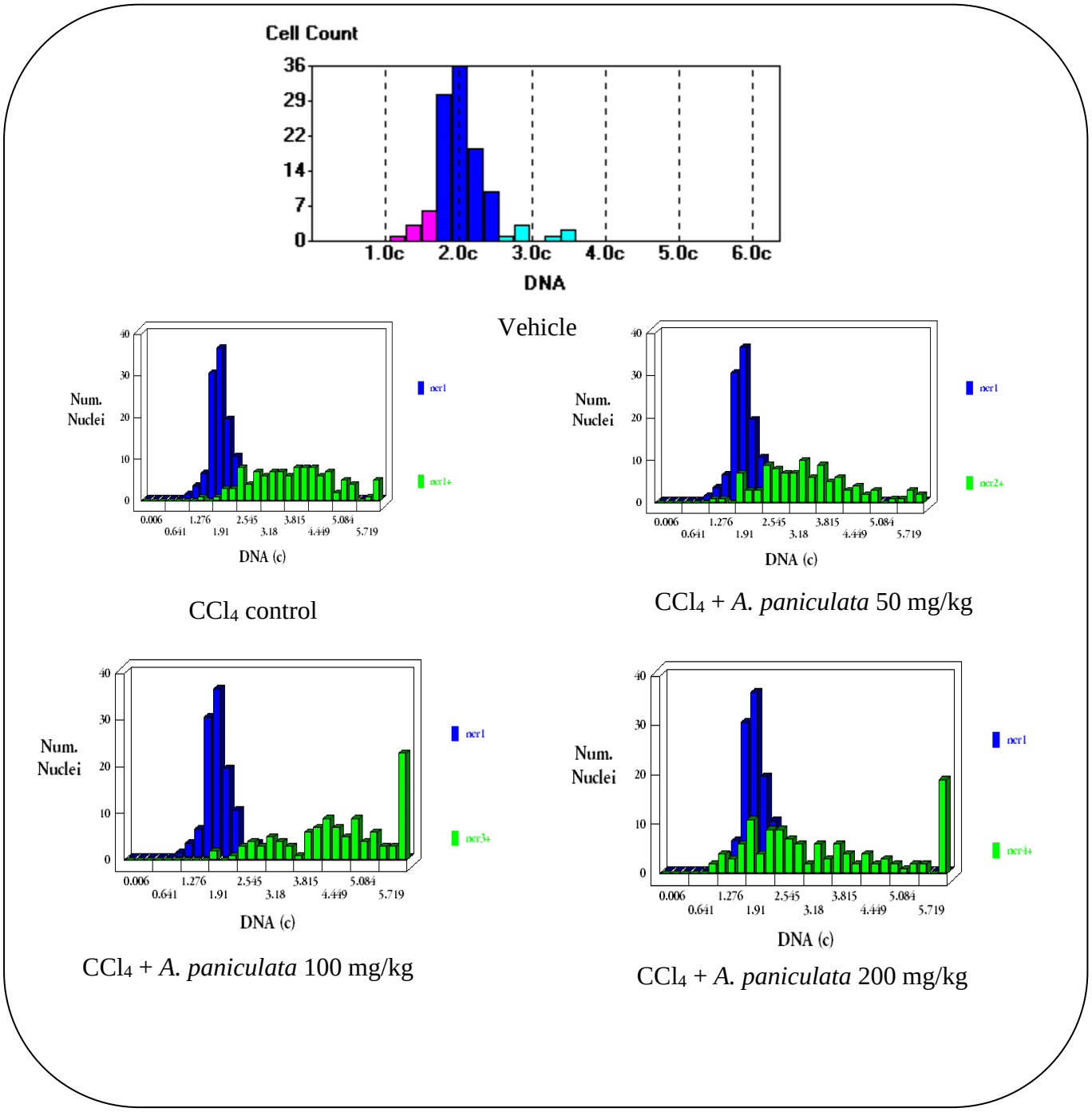

Figure 3 A chart of DNA content in liver cells of rats treated with vehicle, CCl_4 alone or CCl_4 combined with different doses of *A. paniculata*.

Table 1 DNA content of vehicle-treated group.

Range	Total Cells	% Cells	DNA Index	2cDI	DNA MG	Mean	Mode	Std. Dev.	CV	Min	Max
All	112	100.0%	1.000	0.144	0.103	2.060	1.980	0.377	18.307	1.186	3.598
5cER	0	0.0%	-	-	-	-	-	-	-	-	-
< 1.5c	4	3.571%	0.665	0.410	0.262	1.370	1.328	0.135	9.818	1.186	1.471
1.5c - 2.5c	100	89.286%	0.976	0.050	0.037	2.010	1.977	0.224	11.140	1.512	2.498
2.5c - 3.5c	6	5.357%	1.377	0.753	0.429	2.836	2.779	0.254	8.959	2.506	3.224
3.5c - 4.5c	2	1.786%	1.742	2.521	0.961	3.588	3.598	0.014	0.386	3.578	3.598
> 4.5c	0	0.0%	-	-	-	-	-	-	-	-	-

Table 2 DNA content of CCl_4-treated group.

Range	Total Cells	% Cells	DNA Index	2cDI	DNA MG	Mean	Mode	Std. Dev.	CV	Min	Max
All	107	100.0%	1.974	5.682	1.449	4.065	4.167	1.196	29.426	1.571	8.690
5cER	19	17.757%	2.858	15.977	2.161	5.886	5.622	0.959	16.297	5.054	8.690
< 1.5c	0	0.0%	-	-	-	-	-	-	-	-	-
1.5c - 2.5c	7	6.542%	1.051	0.094	0.069	2.164	2.320	0.280	12.955	1.571	2.343
2.5c - 3.5c	28	26.168%	1.448	1.044	0.545	2.982	2.649	0.286	9.575	2.532	3.438
3.5c - 4.5c	36	33.645%	1.941	4.071	1.239	3.997	4.284	0.292	7.294	3.530	4.485
> 4.5c	36	33.645%	2.595	11.988	1.956	5.345	4.871	0.907	16.961	4.505	8.690

Table 3 DNA content of CCl_4 + *A. paniculata* 50 mg/kg-treated group.

Range	Total Cells	% Cells	DNA Index	2cDI	DNA MG	Mean	Mode	Std. Dev.	CV	Min	Max
All	101	100.0%	1.742	3.733	1.186	3.588	2.722	1.106	30.824	1.334	6.936
5cER	10	9.901%	2.827	14.888	2.110	5.823	5.906	0.553	9.502	5.089	6.936
< 1.5c	1	0.99%	0.648	0.443	0.280	1.334	3.308	-	-	1.334	1.334
1.5c - 2.5c	14	13.861%	1.017	0.056	0.041	2.095	2.020	0.224	10.691	1.568	2.474
2.5c - 3.5c	36	35.644%	1.467	1.119	0.573	3.021	2.738	0.278	9.214	2.610	3.491
3.5c - 4.5c	31	30.693%	1.910	3.818	1.200	3.934	3.955	0.281	7.136	3.506	4.421
> 4.5c	19	18.812%	2.581	11.431	1.923	5.315	4.663	0.681	12.805	4.592	6.936

Table 4 DNA content of CCl_4 + *A. paniculata* 100 mg/kg-treated group.

Range	Total Cells	% Cells	DNA Index	2cDI	DNA MG	Mean	Mode	Std. Dev.	CV	Min	Max
All	108	100.0%	2.446	11.912	1.952	5.037	4.356	1.648	32.712	1.924	10.073
5cER	51	47.222%	3.084	20.641	2.346	6.351	5.223	1.321	20.803	5.005	10.073
< 1.5c	0	0.0%	-	-	-	-	-	-	-	-	-
1.5c - 2.5c	3	2.778%	1.041	0.068	0.050	2.144	2.253	0.267	12.445	1.924	2.441
2.5c - 3.5c	16	14.815%	1.467	1.109	0.569	3.021	3.349	0.266	8.805	2.637	3.398
3.5c - 4.5c	23	21.296%	1.994	4.533	1.305	4.107	4.195	0.316	7.700	3.559	4.496
> 4.5c	66	61.111%	2.904	17.640	2.232	5.981	4.734	1.348	22.545	4.509	10.073

Table 5 DNA content of CCl_4 + *A. paniculata* 200 mg/kg-treated group.

Range	Total Cells	% Cells	DNA Index	2cDI	DNA MG	Mean	Mode	Std. Dev.	CV	Min	Max
All	119	100.0%	1.848	7.541	1.637	3.806	2.054	2.077	54.572	1.095	10.249
5cER	27	22.689%	3.396	27.326	2.551	6.994	7.167	1.573	22.488	5.033	10.249
< 1.5c	7	5.882%	0.652	0.447	0.282	1.344	1.402	0.136	10.154	1.095	1.493
1.5c - 2.5c	32	26.891%	1.014	0.070	0.051	2.089	2.084	0.253	12.091	1.635	2.487
2.5c - 3.5c	28	23.529%	1.433	0.994	0.527	2.952	2.611	0.300	10.158	2.553	3.492
3.5c - 4.5c	19	15.966%	1.949	4.138	1.249	4.014	4.024	0.294	7.334	3.563	4.496
> 4.5c	33	27.731%	3.204	23.805	2.450	6.599	7.146	1.655	25.087	4.633	10.249

4. DISCUSSION

The results of this investigation showed that administration of a standardised *A. paniculata* extract could protect against the damaging effects of the potent hepatotoxic compound CCl_4. In this investigation, the treatment of CCl_4 caused noticeably increased serum aminotransferase activity. These enzymes are released into the bloodstream in response to toxic, metabolic, or viral insults to the liver and are considered a reliable indicators of liver damage (Limdi and Hyde, 2003; Yang et al., 2014). As a result, the *A. paniculata* extract's reduction in aminotransferase activity reflects intact hepatic cell integrity.

In CCl_4-treated rats, the effect of *A. paniculata* at 200 mg/kg on lowering serum liver enzymes was equal to that produced by the hepatoprotective drug silymarin. Due to its antioxidant characteristics and effects in stabilising cellular membranes, standardised extracts of silymarin which is derived from the milk thistle plant are frequently employed in the treatment of inflammatory, toxic, and other liver problems (Saller et al., 2001; Gillessen et al., 2022).

The histologic investigation that demonstrated how *A. paniculata* extract inhibited CCl_4-induced liver damage in the form of severe vacuolar degeneration, acidophilic hepatocytes and associated inflammatory cell infiltration further confirmed the herb's favourable liver protective action. The preventive effect of *A. paniculata* extract was unmistakably dose-dependent, with the highest dose of 200 mg/kg resulting in nearly complete liver tissue normalisation. We also demonstrated that along with a high proliferation index, CCl_4 significantly reduced the DNA content of hepatocytes, causing hypoploidy, when compared to the vehicle control group, which is consistent with the results of earlier investigations (Abdel-Salam et al., 2017). At 200 mg/kg, *A. paniculata* significantly reduced the effects of CCl_4 on hepatocyte DNA, which are thought to be a crucial phase in the formation of liver cancer (Weber et al., 2003).

Other researchers who found a protective effect for an aqueous leaf extract of *A. paniculata* on ethanol-induced liver toxicity in the rat (Sivaraj et al., 2011) and of crude methanolic extracts on paracetamol-induced hepatotoxicity in mice (Devaraj et al., 2010) have also confirmed the potential hepatic protective effects of *A. paniculata* extracts. The most prevalent diterpene in *A. paniculata* is andrographolide (Mishra et al., 2007; Raman et al., 2022). It was demonstrated that this active ingredient has anti-inflammatory qualities (Bao et al., 2009). In their study, Cabrera et al., (2017) found that andrographolide could reduce liver triglycerides as well as hepatic fibrosis and inflammation in mice with nonalcoholic steatohepatitis, perhaps through a mechanism involving the suppression of nuclear factor kappa-B (NF-κB).

The generation of the trichloromethyl radical (CCl_3•) by cytochrome P450-dependent monooxygenases is mostly responsible for the harmful effects of CCl_4 on liver cells. This radical can bind to cellular components and cause significant lipid peroxidation and hepatocellular damage. Additionally, there is an increase in the release of nitric oxide and tumour necrosis factor alpha, both of which cause cell damage (Recknagel et al., 1989; Weber et al., 2003). According to research by Zhang and Tan, (2000) and Abdel-Salam et al., (2002), *A. paniculata* extracts contain anti-inflammatory and antioxidant characteristics, which may have contributed to the liver protective effect seen in the current study.

5. CONCLUSIONS

The present study's findings imply that the standardised extract of *A. paniculata* has a protective effect in a rat model of acute liver injury provoked by CCl_4. Thus, *A. paniculata* might prove useful in the management of toxic liver damage.

Author contribution

OMEAS and NS conducted the research and analysis. OMEAS wrote and prepared the manuscript. OMEAS and NS approved the final version of the manuscript.

Informed consent

Not applicable.

Ethical approval

The National Research Centre's Ethics Committee and the American National Institutes of Health's Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) were followed when conducting the experiments. In equal groups of six rats each, the investigation was conducted

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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